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MOLECULAR DOCKING STUDIES OF BETULINIC ACID AND ITS STRUCTURALLY MODIFIED DERIVATIVES AS POTENTIAL INHIBITORS OF COVID-19 MAIN PROTEASE PROTEIN

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ABSTRACT : COVID-19 was announced as pandemic disease in worldwide by WHO on 11th March 2020 and the most common cause of death worldwide now-a-days. However, no exact medication was designed and approved by the FDA yet; many pharmaceutically active compounds have been synthesized to attenuate the incidences of COVID-19 as alternative therapies. To overcome these shaft lines we would like to introduce natural origin products. Betulinic acid (Bet A) is naturally occurring product having a pentacyclic triterpene nucleus with the broad spectrum of biological and pharmacological activities like, antiviral, anti-HIV, antibacterial, anti-inflammatory, anthelmintic, anticancer, antimalarial, activities. SARS-COV-2 consist the main protease protein PDB ID: 6LU7, which plays a potential role in COVID-19 viral replication. Therefore this experiment was designed by software (Autodock tool 1.5.6), Patch dock and Discovery studio 2017 R2 client. Results of molecular docking simulation with 6LU7 and its structurally modified compounds Bet (A1), Bet (A2), Bet (A3), Bet (A4), Bet (A5), Bet (A6), Bet (A7), Bet (A8) and Bet (A9) revealed that Bet (A8) was better binding affinity (-11.53 Kcal/mol) among all modifications including pure Bet A. Bet (A8) interact with GLY 274, LEU 287, MET 276, LEU 286 amino acid and form 2 hydrogen bonds representing most stable and strong complex with 6LU7. Despite this the most recommended potential inhibitors of COVID-19 main protease were Bet (A3) and Bet (A4). Further future research is necessary for the optimization of natural modified compounds.

Key words : COVID-19, natural products, betulinic acid, 6LU7, antiviral.

INTRODUCTION

COVID-19 is most pandemic disease caused by Corona virus (CoVs), which is spreading serious infection in the respiratory tract. In the past (2003 and 2012) decades, two syndrome of SARS-CoV and MERS-CoV, both was transmitted from animals to humans, triggered global epidemics diseases (Paules *et al*, 2020).

In December, 2019 a corona virus disease (COVID-19) was detected in the city of Wuhan, China. The virus was pandemic, declared by the WHO (World Health Organization) and rapidly spread from China to all around 210 countries, on March 11th, 2020 (Cavasotto and Filippo, 2020). This virus was caused by a new pathogenic SARS-CoV-2 (coronavirus-2) and identified as a novel enveloped RNA beta corona virus that phylogenetic similarity aligned to SARS-CoV (Guan *et al*, 2020).

The corona viruses (COVID-19) have been belongs to the beta-family. It is a ssRNA (Single Stranded RNA) virus cantained by lipid membrane and proteins (spike like protein S, Nucleo-Capsid N protein, membrane protein M, and envelope E protein). Protein S is responsible for the adhesion protein onto the ACE2 (Angiotensin Converting Enzyme 2) receptor of lung cells of mammalian, which is release of endogenous viral RNA genetic material into the host cell. 3chymotrypsin protease is the main-protease that is helps in the replication of the virus (Bosch *et al*, 2003; Ge *et al*, 2013 and Han *et al*, 2006).

Patients with the suffer coronavirus from severe acute respiratory infection that may result in death (death range from 0.5–6%) due to alveolar damage and failure of respiratory tract (Xu *et al*, 2020) by the COVID-19 (Lim *et al*, 2020 and Liu *et al*, 2020). According to WHO there is no any specific treatment effective drugs was found against this unprecedented pandemic disease. Some test were approved against to the COVID-19 disease however some protocols like chloroquine, azithromycin and convalescent plasma (Gao *et al*, 2020; Gautret *et at*, 2020 and Chen *et al*, 2020) and management of current disease. It is also work as social measures, like social distancing,

market closed, travel ban and lockdown in many countries in all the world. Thus, there is an urgent need for the finding and discover of drugs and vaccine strategies for the COVID-19.

Betulinic acid (Bet A) is naturally occurring product having a pentacyclic triterpene nucleus with the broad spectrum of biological and pharmacological activities like, antiviral, anti-HIV, antibacterial, anti-inflammatory, anthelmintic, antinociceptive, anticancer, antimalarial, activities (Zhou *et al*, 2020). SARS-COV-2 consist the main protease protein PDB ID: 6LU7, which plays a potential role in COVID-19 viral replication. It is originate in the bark of numerous species of plants, mainly the *Betula pubescens* (white birch) from which it gets its name, *Ziziphus mauritiana*, *Triphyophyllum peltatum*, *Ancistrocladus heyneanus*, *Diospyros leucomelas* (Ji *et al*, 2002) *Tetracera boiviniana*, *Syzygium formosanum* (Zuco *et al*, 2002) and *Pseudocydonia sinensis*. It was also reported work as a selective inhibitor of human melanoma (Pisha *et al*, 1995) and verified to induce apoptosis in human neuroblastoma *in vivo* and *in vitro* condition (Li *et al*, 2020). National Cancer Institute was demostrated and development of drug with assistance from the Rapid Access (Tan *et al*, 2003). Some drugs were showed that against the virus, including lopinavir, oseltamivir, ritonavir, favipiravir, ribavirin, remdesivir, hydroxychloroquine and chloroquine but betulinic acid are most effective against COVID-19 (Li *et al*, 2020; Lim *et al*, 2020; Holshue 2020; Wang *et al*, 2020; Yao *et al*, 2020 and Lv *et al*, 2015).

The therapeutic drugs detection is a time consuming, and very expensive process where in the preliminary screening of large libraries of potential drugs against to the many diseases (Gupta *et al*, 2020 and Sarma *et al*, 2020). With recent study I was found that, molecular docking tools have gained phenomenal acceleration which is lead identification and optimization. These are computational tools facilitate the visualization of the ligand target interaction and identification of target of the compound with efficiency (Zoete *et al*, 2009). The molecular docking are currently available, for widely used is the AutoDock, which is identification, for need to search algorithm and binding modes to perform to the native protein ligand interaction simulations (Sousa *et al*, 2006). This tools helps us in the comprehensive study of intraction and based on bioinformatics and computational biology (Kumar *et al*, 2018).

MATERIALS AND METHODS

Protein

The COVID-19 main protease protein (PDB ID: 6LU7) structure was accomplished from online web server protein data bank (PDB) (https://www.rcsb.org/) in pdb format. PDB ID is utilized to achieve 3-dimensional crystal structures of protein macromolecules (Berman *et al*, 2002). COVID 19 main protease enzymes are of 2 forms; first one is apo (PDB: 6M03) and another is holo (PDB: 6LU7). The holo form (PDB: 6LU7) is more suitable for COVID 19 drug design perspective (Ibrahim *et al*, 2020).

Ligands

Chem Draw 12 utilized for 3-dimensional structure of ligands. Chem Draw 12 is a software providing knowledge on chemical structure and explores their physical and chemical properties.

The natural origin compound betulinic acid (Bet A) used in the present study was triterpinoids (Bet A and its structurally modified compounds Bet (A1), Bet (A2), Bet (A3), Bet (A4), Bet (A5), Bet (A6), Bet (A7), Bet (A8) and Bet (A9) (Kumar *et al*, 2018).

Softwares

Chem Draw 12, Patch dock, molecular docking (Autodock tool 1.5.6), Discovery studio 2017 R2 client.

Patch Dock

Patch Dock is a mathematical based genetic geometrical algorithm for molecular docking. Its principle objective is to ûnd the docking confirmation that produces better molecular architecture complementarity. Molecular docking confirmation is analyzed by a scoring function technique. PatchDock genetic algorithm was accessible at http://bioinfo3d.cs.tau.ac.il/PatchDock. At this web server COVID 19 main protease PDB ID: 6LU7 was uploaded and user email was requisite for the dimensional confirmation notification. Output was mailed as a message to email which consist a URL link of a web page which have top 20 arrangements demonstrating their geometric score, desolvation energy, the actual rigid transformation, the interface area size of the arrangement and a web link to PDB ûle that presents to the docking arrangement (Schneidman-Duhovny *et al*, 2005).

Molecular docking

In silico molecular docking was utilized to anticipate the structure of ligand-receptor complexes. A receptor *i.e.* macromolecule can be a protein or a protein oligomer and the ligand i.e. micromolecule is either a small molecule or another protein. MGL tool supported Autodock 1.5.6 utilized for protein activation, by eliminating water and different molecules, and including a polar hydrogen bond. Further, ligand molecules were activated by central atom selection and make all active bonds in non rotable form.

The gridding was performed to establish the native ligand configuration on the active binding site by arranging the grid coordinates (X, Y, and Z dimensions at 60, 60, 60). Ligand and protein docking was simulated by regulating the Lamarckian genetic algorithm parameter, applying 10 runs of the genetic algorithm criteria. The docking experiment was performed by Autodock tool 1.5.6 and again docked file was analyzed by Discovery studio 2017 R2 client (Kumar *et al*, 2016).

RESULTS

Betulinic acid (Bet A) have active chemical compounds that reported many antiviral activities, which have theoretical probability for binding of protease active site and further serve as inhibitors for covid-19 virus replication. Additionally, FDA-approved antimalarial drugs, Chloroquine and Hydroxychloroquine recommended for treating the COVID–19 patients were used as positive docking controls (Gautret *et al*, 2020). It is a main protease in complex with an inhibitor and docking simulation with 6LU7 and (Bet A and its structurally modified compounds Bet (A1), Bet (A2), Bet (A3), Bet (A4), Bet (A5), Bet (A6), Bet (A7), Bet (A8) and Bet (A9). Bet (A8) having better binding affinity (-11.53 Kcal/mol) among all modifications including pure Bet A. After successful docking of these drugs into the COVID-19 main protease in complex, the results show various modes of binding affinities of betulinic acid (Bet A). Bet (A8) having high affinity interaction (-11.53 kcal/mol) with GLY 274, LEU 287, MET 276, LEU 286 amino acid compared to molecular affinities and it is represented the 2 hydrogen bond, that was showed most stable and strong complex with 6LU7. Drugs were docked against SARS-CoV-2, PDB Id- 6LU7 to analyzed and confirm the efficiencies and potency of antiviral phyto-compounds. Betulinic acid (Bet A) compounds were showed as Bet (A1) (-6.25 Kcal/mol), Bet (A2) (-5.14 Kcal/mol), Bet (A3) (-6.35 Kcal/mol), Bet (A4) (-7.52 Kcal/mol), Bet (A5) (-6.32 Kcal/mol), Bet (A5) (-6.32 Kcal/mol), Bet

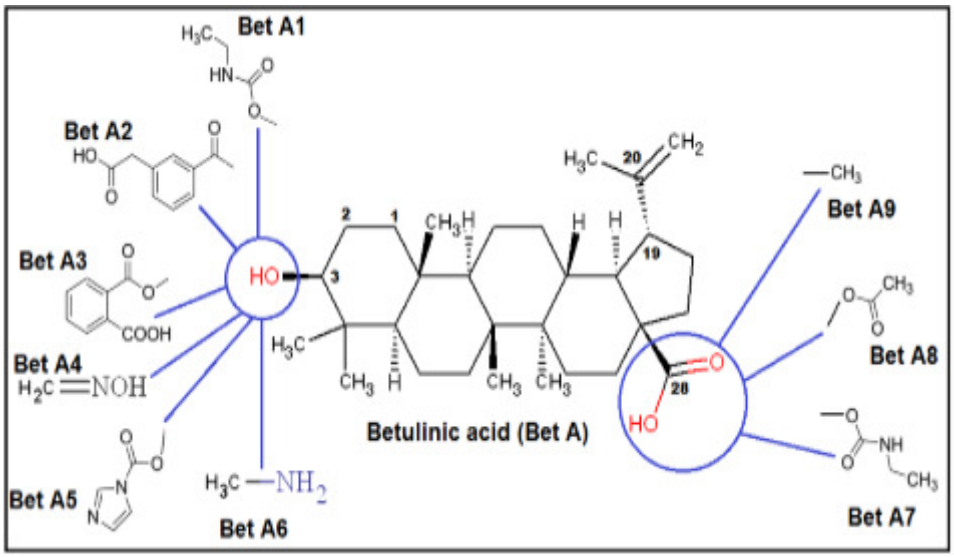

Fig. 1 : Betulinic acid (Bet A) structural modification at C3 and C28 positions.

Table 1 : Binding affinities of Betulinic acid (Bet A) and its derivatives (Bet (A1), Bet (A2), Bet (A3), Bet (A4), Bet (A5), Bet (A6), Bet (A7), Bet (A8) and Bet (A9) with COVID-19, main protease, PDB ID: 6LU7. Comparative studies were performed by AUTODOCK 1.5.4

Lead molecule	Target with PDB	Amino acids involved in Interactions	Affinity (kcal/mol)	H-bonds
Bet A	6LU7	ARG 298, ILE 152, PRO 9, LYS 12, TYR 154	-6.25	1
Bet A1	6LU7	ARG 298, ILE 152, PHE 8, PRO 9, LYS 12, TYR 154	-5.14	1
Bet A2	6LU7	LYS 100, LYS 98, VAL 35, TYR 101	-6.35	2
Bet A3	6LU7	LYS 12, PRO 9, ILE 152, ARG 298, TYR 154	-6.68	1
Bet A4	6LU7	ARG 298, ILE 152, PRO 9, LYS 12, TYR 154	-7.52	1
Bet A5	6LU7	LYS 12, PRO 9, ILE 152, ARG 298, TYR 154	-6.32	1
Bet A6	6LU7	PHE 103, TYR 101, LYS 100, LYS 102	-5.61	1
Bet A7	6LU7	TYR 154, ILE 152, ARG 298, VAL 297, PHE 294	-5.47	1
Bet A8	6LU7	GLY 274, LEU 287, MET 276, LEU 286	-11.53	2
Bet A9	6LU7	LYS 12, PRO 9, ILE 152, PHE 8, PHE 294, ARG 298, TYR 154	-5.27	0

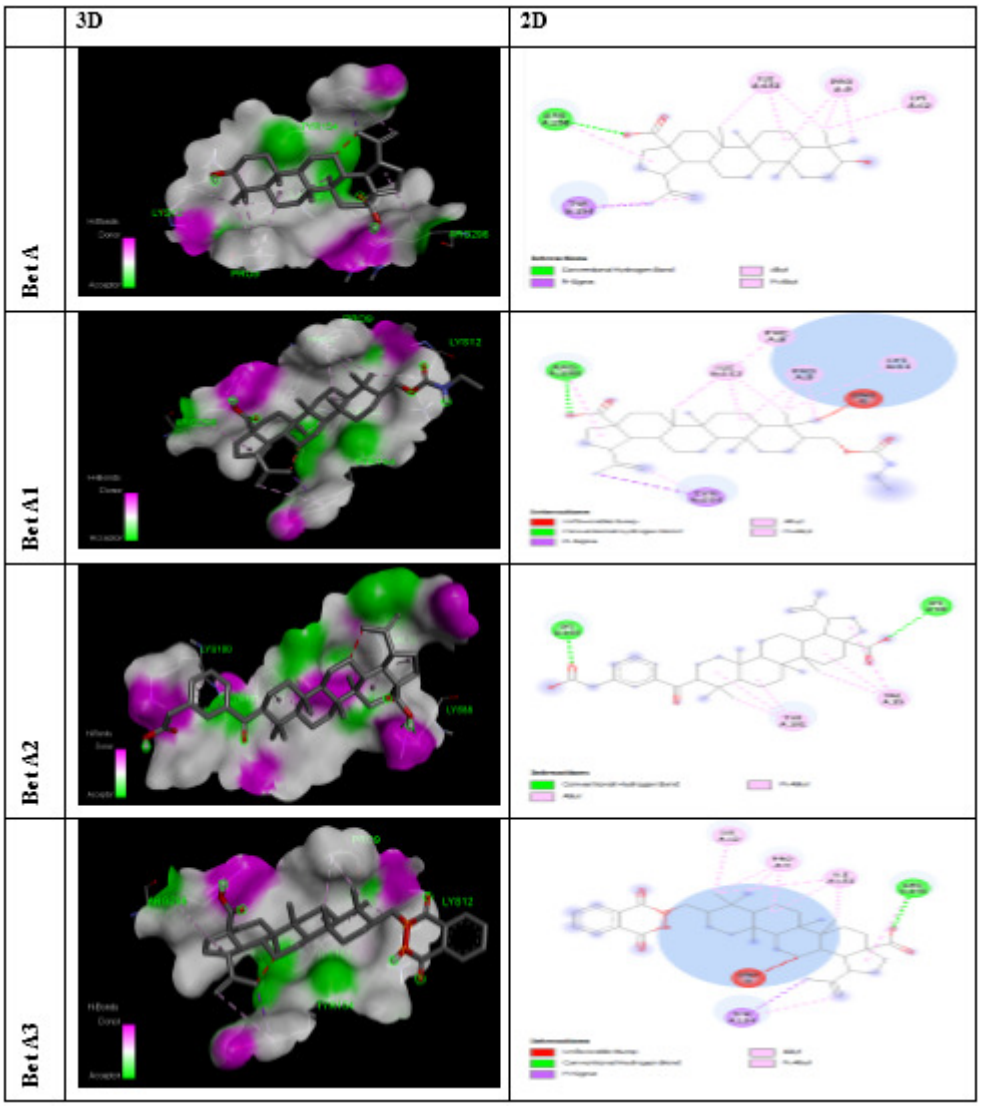

Fig. 2 : Docking poses (3D and 2D) of Betulinic acid (Bet A) and its derivatives (Bet (A1), Bet (A2) and Bet (A3) with COVID-19 main protease, PDB ID: 6LU7. Comparative studies were performed by AUTODOCK 1.5.4.

(A6) (-5.61 Kcal/mol), Bet (A7) (-5.47 Kcal/mol), Bet (A8) (-11.53 Kcal/mol) and Bet (A9) (-5.27 Kcal/mol), that have higher binding affinity in Bet (A4) (-7.52 Kcal/mol) and Bet (A8) (-11.53 Kcal/mol), campare others Bet A compound showed lower binding energy. The antimalarial drug, Betulinic acid (Bet A) was represented as a drug against COVID-19.

DISCUSSION

Betulinic acid (Bet A) is natural drug used as wide range of biological and medicinal properties such as antimalarial, antibacterial, antinociceptive, anti-inflammatory, anticancer activities and antiviral properties. These FDA drugs were used as a comparison, hits and leads (Petrovska, 2012; Ivanova *et al,* 2018; Ding *et al*, 2017; Oliveira *et al*, 2015; Hibasami *et al*, 2004; Gautret *et al*, 2020; Fulda *et al*, 1997 and Ji *et al*, 2002). Wick *et al* (1999) and Thurnher (2003) were also reported that betulinic acid (Bet A) was originated *in vitro* against work also as neuroectodermal (medulloblastoma, neuroblastoma and Ewing's sarcoma), brain tumors, human leukemia HL-60 cells, ovarian carcinoma, carcinoma SCC9 and SCC25 cell lines. Many researcher were also represent the its antiviral properties against some non- enveloped and enveloped viruses (Pavlova *et al*, 2003 and Hong *et al*, 2015). Antiviral activities were defended against several diseases, included hepatitis B, HIV, viruses, influenza virus, and herpes viruses (Khwaza *et al*, 2018 and Oliveira *et al*, 2019). Kumar *et al* (2018) were also reported similar study. Regarding a WHO (World Health Organization) report, 65% to 80% of the population extremely depended on traditional plants for health benefits and 25% of the commonly used drugs and medicines contain compounds isolated from plants (Robinson and Zhang, 2011 and Mukhtar *et al*, 2008). It is very helpful compound against docked in COVID 19,

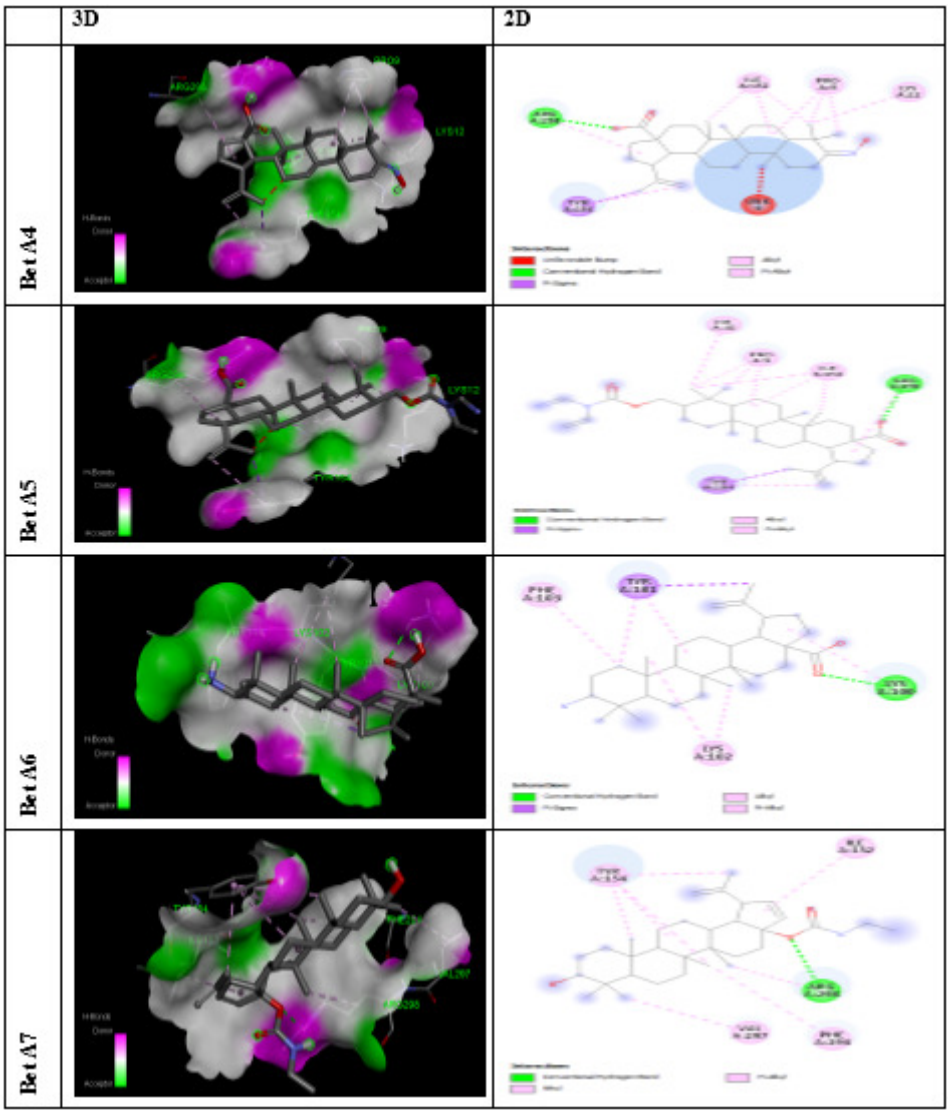

Fig. 3 : Docking poses (3D and 2D) of Betulinic acid derivatives (Bet (A4), Bet (A5), Bet (A6) and Bet (A7) with COVID-19 main protease, PDB ID: 6LU7. Comparative studies were performed using by AUTODOCK 1.5.4.

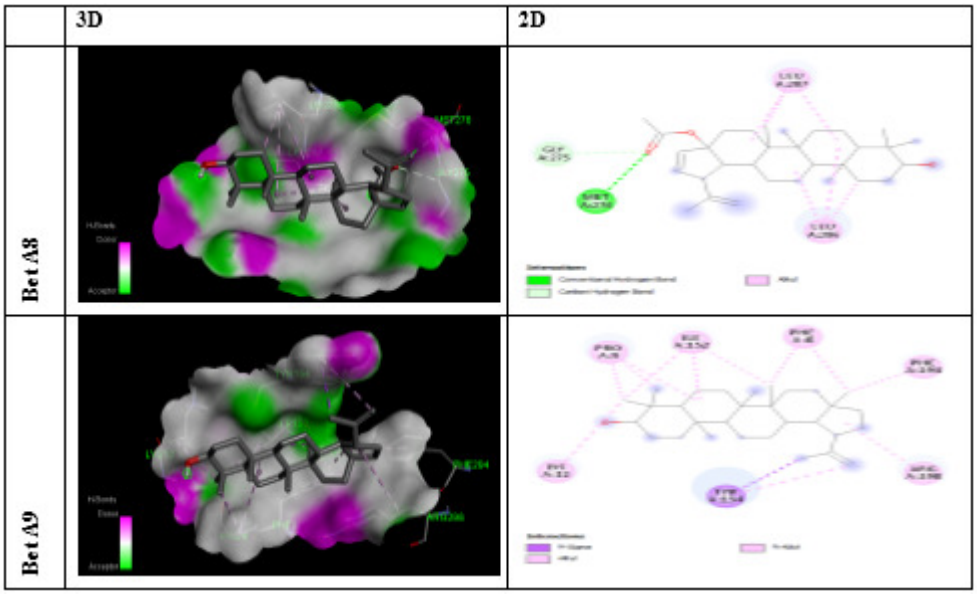

Fig. 4 : Docking poses (3D and 2D) of Betulinic acid derivatives (Bet A8 and Bet A9) with COVID-19, main protease, PDB ID: 6LU7. Comparative studies were performed by AUTODOCK 1.5.4.

main protease in complex with an inhibitor PDB-ID 6LU7. Amino acid residues involved in interaction and bind of compounds with amino acid with hydrogen bond (Narkhede *et al*, 2020). The compound interacted with amino acid residues in the active site of the protease with 2 higher hydrogen bondings within 3 Å with 7 amino acid residue (Khan *et al*, 2020 and Shivanika *et al*, 2020).

Molecular docking study of betulinic acid (Bet A) with both COVID-19 main protease in complex with an inhibitor 6LU7 and also worked on SARS Spike glycoprotein-Human ACE2 complex attributed many facts about the binding affinity (Khodarahmi*et al*, 2015) and number of hydrogen bond, the higher the binding efficiency and inhibition (Kumar *et al*, 2015). It also showed pi-pi and pi-alkyl interaction with residues of VAL 35, PRO 9 and ILE 152. Similar to chloroquine, hydroxychloroquine also showed promising interaction with the SARS Spike glycoprotein-Human ACE2 complex with the binding affinity of -6.8 kcal/mol (Narkhede *et al*, 2020). This interaction of amino acids were involved in vander Waals interaction due to better understanding and fitting inside the protein. Muralidharan *et al* (2020) also reported the molecular docking in-silico of lopinavir, oseltamivir and ritonavir against the 6LU7 using AutoDock and represented the binding energy of -4.10 kcal/mol, -4.65 kcal/mol and -5.11 kcal/mol respectively, and amino acids in the active site *viz*., GLN 189, GLU 166, HIS 41, MET 165 and PRO 168 (Muralidharan *et al*, 2020). Wen *et al* (2007) reported that savinin and betulinic also were worked againt SARS-COV-1. Mirza and Froeyen (2020), performed similar study, docking of 6LU7 of SARS-CoV-2 and observedcovalent interactions between proteases (CYS 145 and HIS 41). Similar study found that the study of CYS 145 and HIS 41 amino acids in the active site withthe peptide inhibitor (Andrade *et al*, 2020). This partially proved the efficiency and validityof the docking protocol also reported by Joshi *et al* (2020). Many researchers also represent many theories against work on COVID-19 (Yu *et al*, 2020; Lalani and Poh, 2020: Liu *et al*, 2020 and Singh *et al*, 2020).

CONCLUSION

COVID-19 was declared as a pandemic by WHO on March 2020 worldwide. Conversely no affirmed drug is as of now accessible for exact medication. Researchers fundamentally target the main protease to cure COVID-19 infection. In the current article, we examined inhibitory activity of Bet A and its structurally modified derivatives (Bet (A1), Bet (A2), Bet (A3), Bet (A4), Bet (A5), Bet (A6), Bet (A7), Bet (A8) and Bet (A9) on COVID-19 main protease. The result of this study showed that Bet (A4) and Bet (A8) have better binding affinity among all modifications including pure Bet A. The most recommended molecule was Bet (A8) (modification at C28 position with more polar group as potential inhibitor of COVID-19 Main protease). Future exploration may broaden this work by introducing in vitro, in vivo clinical experiments and if the simulation results approved, Bet A modified compounds might be considered to work against in COVID-19.

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